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A STUDY OF TYPHOID OPSONINS.*

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THE investigations which have been made in regard to the opsonins arising either during the course of typhoid fever, or subsequent to the inoculation of animals with living or dead cultures of typhoid bacilli, have not led to uniform conclusions. Wright and Douglas[1] found that the typhoid bacillus is highly susceptible to the opsonic action of serum; they class typhoid fever, however, with those diseases which give a constantly low opsonic index.

Leishman, Harrison, Smallman, and Tulloch[2] came to the conclusion that opsonin, if present in typhoid fever, cannot be demonstrated by the technic employed by them.

H. Klien[3] reports the results of a thorough study of the opsonins arising subsequent to the subcutaneous injection of living typhoid bacilli into rabbits. The results which he obtained by the use of Wright's original method of determining the opsonic index would indicate that the index after such injections does not rise very high. Klien, however, placed little confidence in the results obtained by this method,

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* Received for publication December 10, 1907.

[1] *Proc. Roy. Soc.*, 1904, 73, p. 136.

[2] *Jour. Hyg.*, 1905, 5, p. 380.

[3] *Bull. Johns Hopkins Hosp.*, 1907, 18, p. 245.

and on that account he conducted his later examinations on a different method. He determined the point of dilution at which phagocytic action was sufficiently great to give a phagocytic index of 0.5. This degree of dilution was taken to represent the degree of opsonic activity. From his study of typho-opsonins with this method Klien concludes unreservedly that "it has been shown that by immunizing a rabbit against typhoid, opsonins are formed in a considerably high degree."

Klien also found that agglutinins, opsonins, and lysins increase at the same rate after the injections, but that later the opsonins and lysins increase more rapidly than the agglutinins; the rates of increase in the former may be identical; furthermore, that immune opsonin is thermostable, resisting heating at 55–58° C. for 30 minutes, and that normal opsonin is thermolabile but yet does not disappear entirely after exposure to a temperature of 56–58° C. for 30 minutes.

Neufeld and Hüne[1] regard the opsonic action of normal serum as due to amboceptor and complement, which cause not complete death of the bacteria acted upon, but a certain amount of injury which renders them susceptible to phagocytosis; whereas immune opsonins are held to be distinct new substances which resist the action of heat. They acknowledge, however, that sometimes undiluted normal serum after being heated to 56° C. for 30 minutes favors phagocytosis of certain bacteria. But in this case they hold the phenomenon to be unexplained. They found that the serum from a patient with paratyphoid fever and sera of animals immunized with paratyphoid bacilli may contain opsonin, or tropic substances, as they say, but no lysin for paratyphoid bacilli. Such sera may, however, contain lysin for typhoid bacilli.

METHOD.

The plan pursued in our investigations is essentially that used by Wright and his followers in the determination of indices for other organisms, and may be considered under the following headings:

Washed leucocytes.—These are obtained by collecting in 0.85 per cent sodium-citrate solution blood from the lobule of the ear. The leucocytes and erythrocytes are now precipitated by centrifugal force and subsequently washed once or twice with physiological salt solution. The "cream" is then drawn off and used in the experiment.

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[1] *Centralbl. f. Bakt.*, Abt. 1, 1908, 38, Ref. p. 28; *Arb. a. d. kais. Gesund.*, 1907, 25, p. 1.

Bacillary suspension.—A 24-hour broth culture of the typhoid bacillus is diluted to a moderate cloudiness; otherwise spontaneous phagocytosis will most likely interfere with the results.

Typhoid bacilli even when recently isolated may give considerable spontaneous phagocytosis while, so far as observed, fresh paratyphoid bacilli give practically none at all. Repeated transplantation may render a typhoid strain originally not subject to spontaneous phagocytosis quite markedly phagocytable without the intervention of serum. The 24-hour broth cultures of typhoid bacilli are much denser than those of paratyphoid bacilli, and we found that, as a rule, dilution of typhoid broth cultures so that the density approximates the undiluted paratyphoid culture obviates spontaneous phagocytosis almost completely. While before dilution there may be spontaneous phagocytosis of from ½ to 1 bacillus per leucocyte and even more, dilution reduces this to nil or practically nil (0.018, 0.002, etc.). The most useful suspensions are those giving an average phagocytosis of 1.0–1.5 bacillus per leucocyte with heated normal serum; if suspensions of greater density are used it may be impossible to determine the index of highly opsonic immune sera because the number of bacilli taken up under their influence may be so great that it is impossible to count them.

When a recently isolated paratyphoid organism is used, dilution is not necessary since this organism, as a rule, grows much less luxuriantly than the typhoid bacillus, and spontaneous phagocytosis is either slight or entirely absent. Old cultures, however, may show spontaneous phagocytosis.

Serum.—Blood is collected from the ear into a small U-tube which is centrifugated; the supernatant serum is employed in the experiment. The normal control serum consists of a mixture in equal parts of sera derived from three or more persons.

Because of the marked lytic effect of both normal and typhoid sera, it has been our practice to heat the sera to 56° C. for 20–30 minutes in order to destroy the typhoid complement and thus be able to compare the effect of the thermostable opsonic substances in different sera. We have found that normal human and normal rabbit serum always retain a distinct opsonic effect upon typhoid and paratyphoid bacilli after inactivation at 56° C.

Mixture of elements.—Equal parts of washed leucocytes, heated serum, and bacillary suspension are thoroughly mixed in a capillary pipette, and the mixture incubated at 37° C. for 30 minutes.

Estimating the index.—Smears are made in the usual way, stained with carbolthionin and the number of organisms in from 40 to 100 leucocytes counted. The average number of bacilli taken up by a given number of leucocytes in typhoid or paratyphoid serum is divided by the average number of bacilli taken by as many leucocytes in the normal serum and the quotient is the opsonic index. Inasmuch as the patient's serum as well as the normal control serum are both heated, it is consequently the thermostable opsonins in both that are determined and compared quantitatively.

DETERMINATIONS OF OPSONIC INDEX IN TYPHOID FEVER.

We have found that there is a rather wide variation in the number of bacilli taken up in various normal sera even though the same technic be followed rigidly. Furthermore, the serum of an individual may be highly opsonic for the same organism one day and very moderately so the next, as shown by the following results obtained on two days with sera from four normal individuals:

	May 6	May 10
1	54	58
2	47	100
3	24	246
4	186	62

It is also a striking fact that various strains of typhoid bacilli give different indices with any given typhoid serum. This fact was also observed by Klien. So marked is this difference that we cannot define any given course as the one representing the typic opsonic variation in typhoid fever. We have even met with one strain that gave a persistently low index as compared with other strains. The general course may, however, be the same with many strains. Charts 1 and 3 show the index obtained for the bacillus from the patient's blood in a case of typhoid fever. Further observations are desirable.

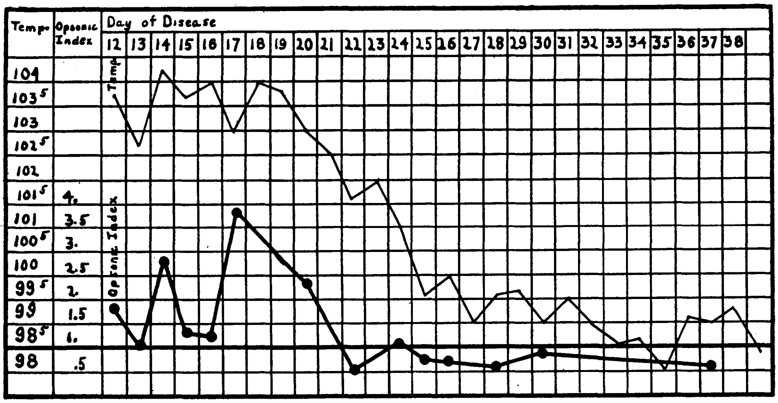


CHART 1.—Typho-opsonic index for bacillus from patient's blood in typhoid fever.

We have frequently noted that a recently isolated organism will be taken up readily in immune serum, but only to a slight extent in normal serum. After a variable number of transplantations on various media the organism may become readily susceptible of phagocytosis in both normal and immune sera. As an example of this are given the figures obtained on different occasions with a bacillus isolated from the blood of a typhoid-fever patient: On April 23 the patient's serum gave an average phagocytosis of 1.25, while the normal gave only 0.06; on April 25 the immune gave 6.6, the normal 1.85; on April 30 the immune gave 1.45 and the normal 0.93. Here phagocytosis was marked in the immune serum from the beginning, but was at first very weak in normal serum. In this and other similar experiments very special care was taken in order to secure suspensions of the same density.

The indices obtained for staphylococcus, streptococcus, diphtheria bacillus, pneumococcus, and tubercle bacillus with immune serum rich in opsonin for typhoid bacilli are not correspondingly high, but are as a rule normal. There is usually obtained, however, a high index with paratyphoid bacillus. In fact it has been our experience that the opsonins arising in the course of typhoid fever are generally if not constantly equally capable of stimulating phagocytosis of the paratyphoid bacillus.

We have found, too, that while the index obtained in typhoid fever with the typhoid bacillus may not be high at a given time, the index obtained with the paratyphoid bacillus at the same time is often very high. This high index may be obtained with several paratyphoid strains. Thus in 12 examinations of blood from various typhoid cases in which the indices were obtained at the same time with three strains of paratyphoid, and four of typhoid bacilli, the four organisms giving the highest average indices were three paratyphoid and one typhoid strain.

In the cases in which we examined the serum with one paratyphoid strain the index was found uniformly high early in the disease, approaching normal as convalescence set in, often rising again during the first part of convalescence, again to approach normal during its close. In one case a relapse occurred and there was a repetition of the characteristic curve (see Chart 2).

As stated, various strains of typhoid and paratyphoid bacilli may give different indices with the same serum. Although the rise of the index with one strain is not necessarily followed by a rise in the index with other strains, yet one often sees a corresponding rise and this rise may be quite accurately proportional as shown in Table 1. Here it is seen that during the course of a month, in a typhoid patient, the relation of the index with the paratyphoid bacillus "P" to that obtained with

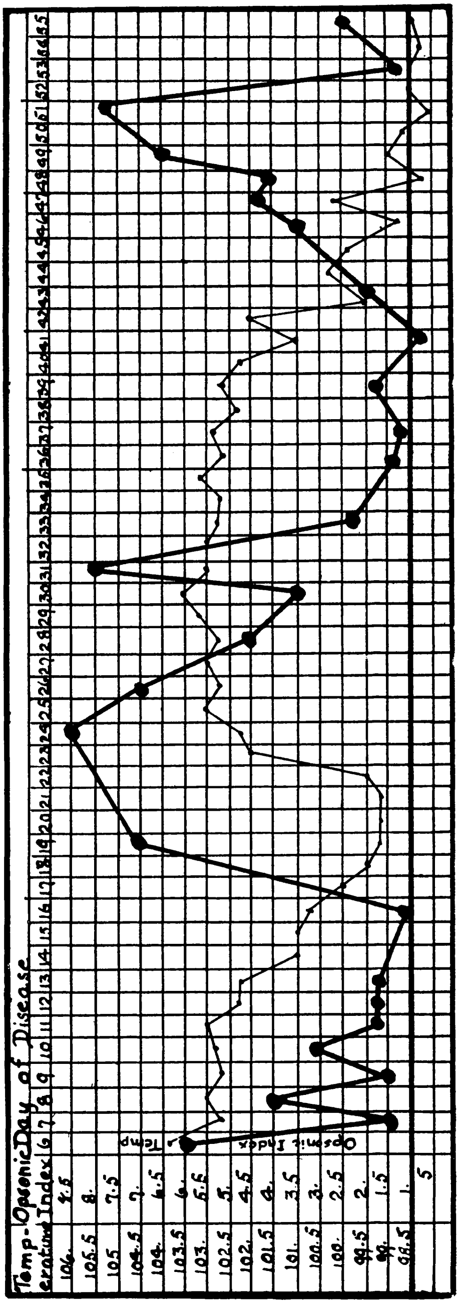


CHART 2.—The opsonic index in typhoid fever for paratyphoid bacillus.

TABLE I

DATE	BACILLUS	INDEX	—PROPORTION—		
			P/M	P/G	G/M
Jan. 6	P	7.00			
	M	0.69	10.1		
" 10	P	5.88			
	M	0.51			
	G	1.74	11.5	3.3	3.4
" 12	P	3.11			
	M	0.29			
	G	0.96	10.7	3.3	3.3
" 20	M	0.21			
	G	0.63			
" 24	P	3.4			3.0
	G	1.11		3.1	
Feb. 10	P	6.9			
	M	0.75	9.2		

P = Paratyphoid
M = Typhoid
G = Typhoid

the typhoid bacillus "M" was approximately 10 to 1; this, regardless of the fact that one was a high index, the other uniformly subnormal. The relation of the index of bacillus "G" to that of "P" and "M" was 3 to 1, This relationship is not infrequently observed.

In a case of typhoid fever several examinations were made by the dilution method used by Klien at the same time as the index was determined in the usual way (Chart 3). The patient's own organism was used; the results obtained by the two methods in this case agree very well. We regret that more observations of this kind have been impossible up to the present time. It is strongly suggested by the results thus far obtained, however, that the general outlines of the opsonic curves obtained by the two methods will be the same, assuming, of course, that heated serum be used and an organism that does not give spontaneous phagocytosis.

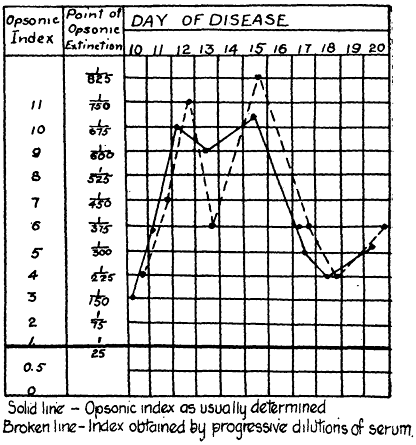
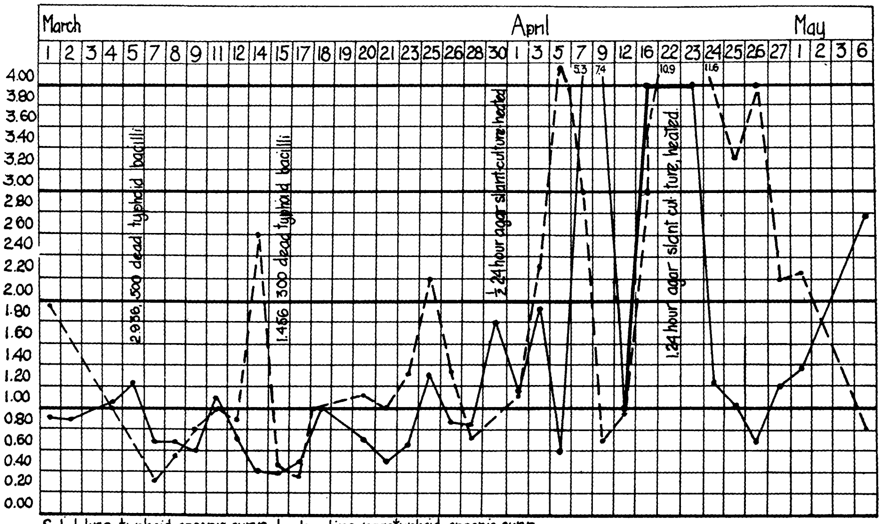


CHART 3.—Comparison of opsonic index for bacillus from patient's blood in typhoid fever as usually determined and as obtained by progressive dilutions of the serum.

In most of the cases studied the diagnosis of typhoid fever was established by the isolation of the typhoid bacillus from the patient's blood.

THE FORMATION OF SPECIFIC OPSONINS AFTER INJECTION OF RABBITS WITH KILLED TYPHOID AND PARATYPHOID BACILLI.

A study of the opsonins in rabbits following inoculations of dead cultures of the typhoid and paratyphoid bacilli brought forth certain definite conclusions. Preliminary to the experiments the indices of three normal rabbits were estimated on five successive days in the usual manner, so as to obtain some idea of the normal variations. For the typhoid bacillus the normal maximum and minimum appear to be 1.3 and 0.7 respectively. In the estimation of the index with the paratyphoid bacillus a greater variation was found, since normal rabbit serum appears to possess only a moderate amount of opsonin for this organism only a slight increase or decrease in the paratyphoid-opsonic content of a single serum to be tested may produce quite considerable differences in the opsonic index from day to day. For this organism the maximum index was 1.97; the minimum, 0.7; and the



Solid line, typhoid opsonic curve; broken line, paratyphoid opsonic curve.

CHART 4.—Opsonic indices for typhoid and paratyphoid bacilli in rabbit injected with killed typhoid bacilli.

average of the five estimates, 1.32. There was not any close correspondence between the typhoid and paratyphoid opsonic index in the same rabbit on the same day. For example in rabbit A the typhoid opsonic index was 0.96; the paratyphoid index at the same time was 1.97.

The observations on the injected animals extended over a period of 67 days. In the beginning the opsonic index of each rabbit to both the typhoid and paratyphoid bacillus was taken daily; later at less frequent intervals. The serum of three normal rabbits, collected at the same time as the immune serum, was used as the normal standard. The serum was heated for 15 to 20 minutes at 56 to 58° C. Human leucocytes were used as phagocytes. Otherwise the Wright technic was followed.

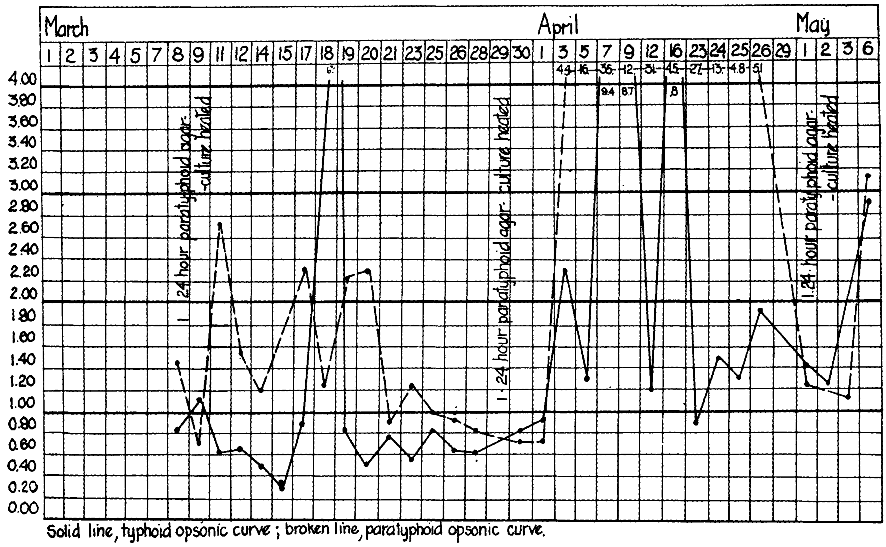
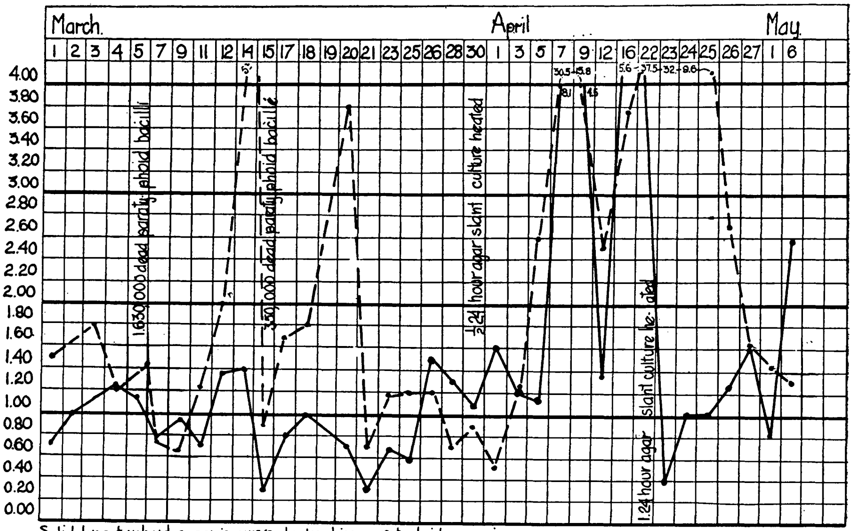


CHART 5.—Opsonic indices for typhoid and paratyphoid bacilli in rabbit injected with killed paratyphoid bacilli.

Subcutaneous injections of suspensions of killed bacteria from 24-hour agar slant growths of one typhoid and two paratyphoid strains were given at intervals of 10 days or more. Rabbit A received one 24-hour agar slant growth of *B. typhosus* (strain 8) suspended in sodium-chloride solution and heated to 66° for two hours. Rabbit B received a 24-hour agar slant growth of both *B. typhosus* (strain 8) and *B. paratyphosus* (strain 12) similarly treated; rabbit C received a similar dose of paratyphoid bacilli (strain 12), and rabbit VI received a like amount of paratyphoid strain 6. Charts 4 to 7 show the effect on the opsonic index (to strains 8 and 12) of these injections.

It is to be noted, first of all, that the injections of either typhoid or paratyphoid baclli cause a rise in the opsonic index for both organisms. This is in accord with the facts obtained in the study of the opsonin of typhoid patients, namely that at certain stages of the disease the patient's serum gives high indices with both organisms.

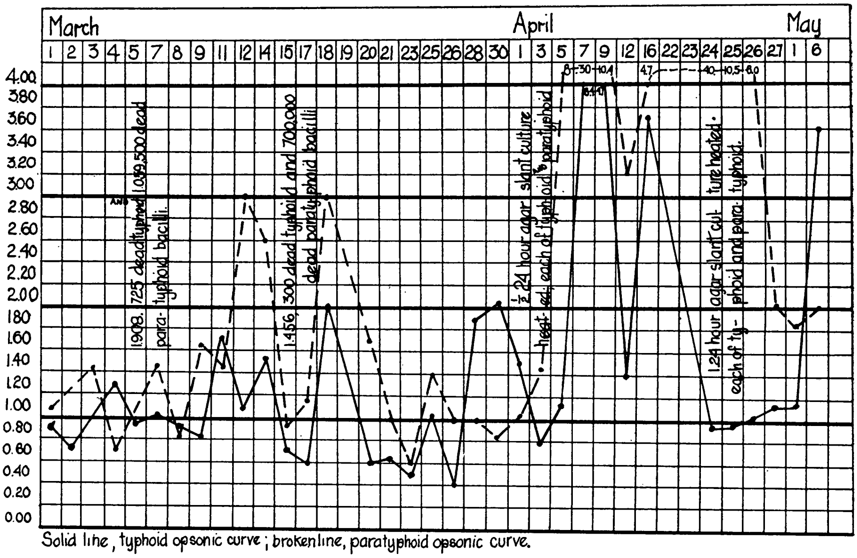


Solid line, typhoid opsonic curve; broken line, paratyphoid opsonic curve.

CHART 6.—Opsonic indices for typhoid and paratyphoid bacilli in rabbit injected with killed paratyphoid bacilli.

One experiment also indicates that the serum of rabbit A possessed a high opsonic index to dysentery bacilli (Shiga and Flexner). There is reason, however, to believe that the opsonins for typhoid and paratyphoid bacilli in these sera, although they contain largely a common element, are, in the main, separate and distinct bodies.

In the first place the curves do not always move in the same direction. Thus, the paratyphoid index may be low when the typhoid index is high, and vice versa. As a rule, however, their general trend is the same, at least sufficiently so to indicate that both opsonins are produced from the stimulus of the same injection. In the second place immune rabbit sera treated with large quantities of typhoid bacilli show greater diminution in the amount of typho-opsonin than of paratypho-opsonin and vice versa. In these absorption experiments there was used in each case the same amount of serum and of bacillary suspension or of salt solution (control); after an exposure for 60 minutes to 37° C. all the mixtures were filtered, including those of serum and salt solution, and the ordinary phagocytic experiment was now carried out by mixing equal parts of serum, leucocytic suspension, and bacillary suspension. The actual results are shown in



Solid line, typhoid opsonic curve; broken line, paratyphoid opsonic curve.

CHART 7.—Opsonic indices for typhoid and paratyphoid bacilli in rabbit injected with killed typhoid and killed paratyphoid bacilli.

Table 2, and they plainly indicate that the serum contains two distinct opsonic bodies. It must be noted that the paratyphoid opsonin produced after injection with typhoid bacilli is partially absorbed by typhoid bacilli; also that the typhoid opsonin produced by inoculation with *B. paratyphosus* is partially absorbed by *B. paratyphosus*.

Contrary to the results obtained with dead streptococci, there appears to be a gradual increase in the rise of the opsonic index after each successive injection. Thus the first injection of rabbit A was followed by a marked negative and almost no positive phase; whereas after the second and third injections the negative phase became less

TABLE 2

The Effect of Treatment of Antityphoid and of Antiparatyphoid Rabbit Sera with Typhoid and Paratyphoid Bacilli.

Serum	Average No. of Bacilli per Leucocyte	
	Typhoid Bacilli	Paratyphoid Bacilli
Antityphoid Serum untreated	7.8	1.0
" " treated with Typhoid Bacilli	1.0	0.5
" " " " Paratyphoid Bacilli	7.4	0.36
Antiparatyphoid Serum untreated	3.0	2.0
" " treated with Typhoid Bacilli	2.0	2.0
" " " with Paratyphoid Bacilli	1.0	0.016
Nace Solution only	0.016	0.015

marked and the positive much greater. The enormous indices obtained after the third injection are interesting, especially when we note that they remained high for a considerable length of time.

The opsonins that develop after injections of typhoid and paratyphoid bacilli do not affect the index to the tubercle bacillus; and usually a given vaccine produces a greater rise in the opsonic index toward the organism injected than to the other of the organisms used in these experiments. The virulence of the typhoid strain seems not only to affect the amount of phagocytosis but curiously enough also the opsonic index obtained. Strain 8′ in the table is a two-month-old agar culture of strain 8; strain 8 is the parent organism used in these experiments and has been transferred to fresh broth daily; strain 8″ is strain 8 modified by being passed through a series of 12 guinea-pigs, the last pig being killed by ¼ c.c. of a broth culture injected intraperitoneally. The experiment was done with the same serum, on the same day, under exactly the same conditions throughout and with suspensions of the organisms which appeared of exactly the same

TABLE 3

The Effect of Virulence of the Strain upon the Opsonic Index.

Strain	Serum of Rabbit A		Serum of Rabbit B		Serum of Rabbit C		Serum of Rabbit VI	
	No. of Bacilli in 50 Leucoc.	Index	No. of Bacilli in 50 Leucoc.	Index	No. of Bacilli in 50 Leucoc.	Index	No. of Bacilli in 50 Leucoc.	Index
8′	65	1.02	78	1.21	72	1.13	63	0.98
8	..	0.83	..	1.94	..	1.29	..	0.62
8″	17	0.46	23	0.62	32	0.86	23	0.62

density. It is seen that the opsonic index obtained with the virulent organism is lower in each serum than that with the less virulent strains. At present we offer no explanation of this phenomenon.

SUMMARY.

After heating normal human and rabbit serum to 56–58° C., there persists in these sera in appreciable degree the power to promote phagocytosis of typhoid and paratyphoid bacilli by washed human leucocytes. As the result of spontaneous infection as well as artificial inoculation with these bacilli this power of the serum may undergo profound modifications and may be augmented in very high degrees. The exact determination of this opsonic power with respect to typhoid bacilli in the course of typhoid fever was found to present many difficulties on account of inconstancy and variations in the induced and spontaneous phagocytability of the typhoid bacillus, demanding much further study, whereas with respect to paratyphoid bacilli the variations of the opsonic power of the serum of typhoid-fever patients are more easily determined and appear to follow a fairly typical course, the opsonic index as determined by comparison of heated normal and typhoid sera, being high early in the disease when the patients ordinarily come under observation, approaching normal more or less gradually as convalescence sets in. In rabbits injected with either dead typhoid or dead paratyphoid bacilli there develop in each case immune opsonic substances for both these organisms; these substances, however, appear to be largely specific, no matter whether produced in response to the injection of the one or the other of the bacteria.